A new technique for worm burden assessment of Angiostrongylus vasorum in experimentally infected foxes (Vulpes vulpes)

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New technique for worm burden assessment of *Angiostrongylus vasorum* in experimentally infected foxes (*Vulpes vulpes*)

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Fig. 1. Prevention of post mortal blood clotting: Intravenous injection of heparin (350 IU / kg).

Fig. 2. Euthanasia: Intravenous injection of pentobarbital (100 mg / kg).

Fig. 3. Blocking the natural outlet from left ventricle: Clamping the aorta (arrow) proximal to the carotid arteries.

Introduction

Assessing the *Angiostrongylus vasorum* worm burden of a host animal is very complicated due to its very delicate location in the peripheral branches of the pulmonary arteries. So, in field surveys approximate quantification is usually done by dissecting the pulmonary arteries and lung tissue, resulting in a high degree of worm damage and subsequent identification problems.

Perfusion technique

In a large-scale angiostrongylosis study including 75 experimentally infected foxes, a novel technique for reverse perfusion of the cardio-pulmonary vascular system was developed in order to recover as many intact worms as possible. Anaesthetized foxes were given heparin i.v. (350 IU/kg) in order to prevent blood clotting during *post mortem*. Three minutes later a lethal pentobarbital dose (100 mg/kg) was given, the thorax was opened and the thoracic organs were perfused *in situ*. The aorta, vena azygos and both venae cavae were clamped off. About three litres of isotonic perfusion liquid (sodium citrate, 15 g/l + NaCl, 8.6 g/l dissolved in tap water) were pumped via a 16G needle into the left auricle, through the pulmonary veins, the lung capillaries and the pulmonary arteries to the pulmonary trunc from which it was led via a plast pipe (5 mm diameter) onto a fine sieve (200 µm aperture) for collection of worms. This procedure was followed by removal of the lungs, dissection of the pulmonary arteries and subsequent baermannization of the chopped lung tissue in normal saline. The detailed procedures are shown in figs. 1-10.

Results

By estimating the total worm burden as the sum of recoveries made by perfusion, dissection and baermannization, respectively, 59%, 28% and 13% of the total worm burden (3,311 worms) were recovered by each of these three methods, respectively (see fig. 11). In contrast to dissection and baermannization, however, close to 100% of the worms recovered by perfusion were intact.

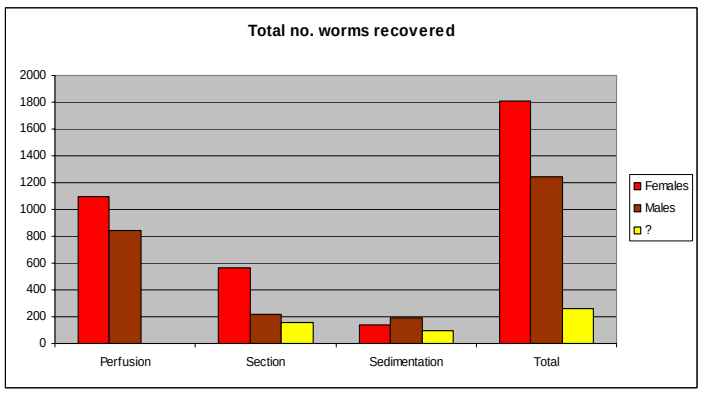

Fig. 11. Worm recoveries resulting from perfusion, dissection (section) and Baermann techniques, respectively, applied in a group of experimentally infected foxes.

Conclusion

There is no golden standard technique for quantification of adult *A. vasorum* worm burdens. Thus, despite the new perfusion technique is superior for isolating intact worms, optimal worm burden assessment may only be achieved in combination with the two traditional (and laborious) techniques, i.e. comprehensive dissection of the pulmonary arterial branches followed by chopping and Baermann sedimentation of the remaining lung tissue.

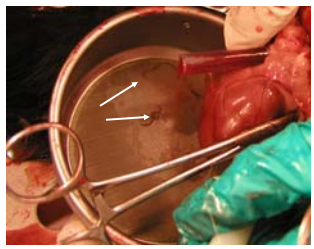

Fig. 10. Recovery of heart worms from pulmonary arteries: Trapping intact adult *A. vasorum* worms (arrows) on a sieve.

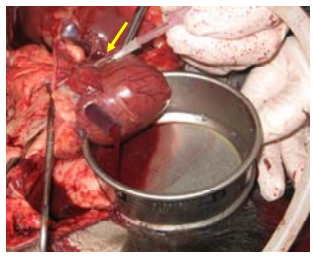

Fig. 9. Reverse perfusion of pulmonary vascular system: Pumping perfusion fluid into left atrium (arrow) via pulmonary veins and arteries to pulmonary trunc; outlet via plast pipe.

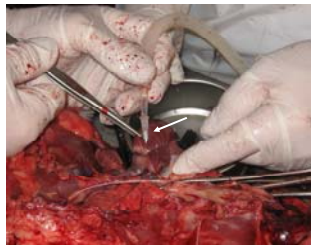

Fig. 8. Placing the perfusion inlet to the left atrium: Insertion of a 16G needle (arrow) into the left atrium .

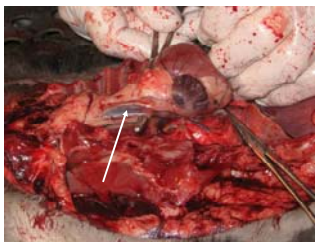

Fig. 4. Preventing anterior flow from the right atrium: Clamping the anterior vena cava (arrow).

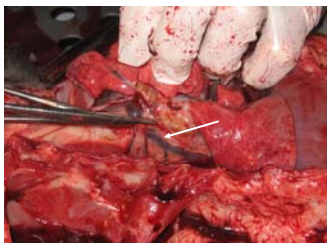

Fig. 5. Preventing dorsal flow from the right atrium: Clamping the the azygous vein (arrow).

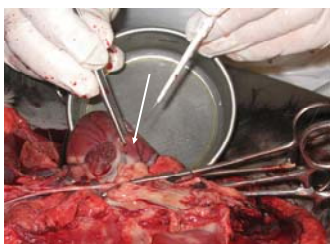

Fig. 6. Making an opening to the pulmonary trunc: Incision (arrow) into the right ventricle near the pulmonary trunc.

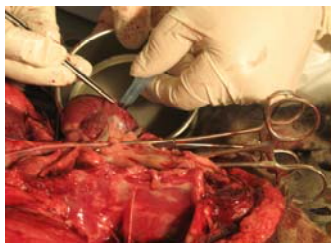

Fig. 7. Perfusion outlet from the pulmonary trunc: Placing a plast tube to keep the pulmonary valves open during perfusion.